Fig: S1A

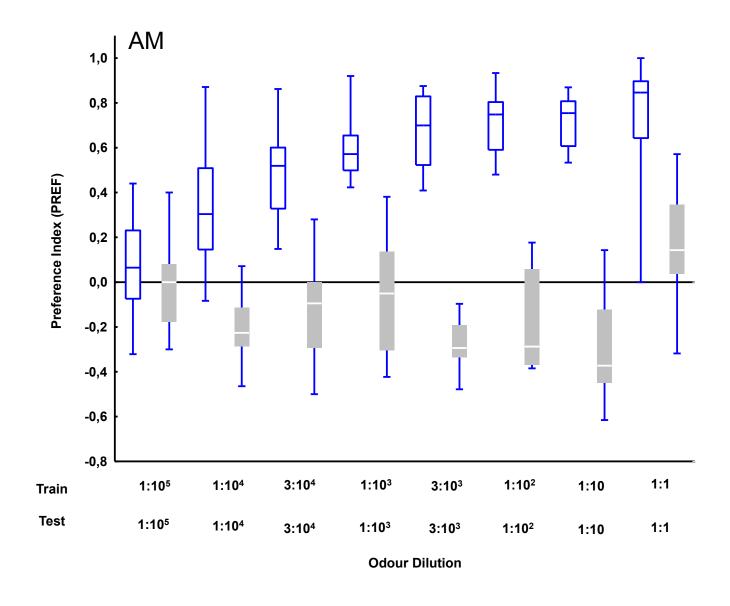


Fig: S1B

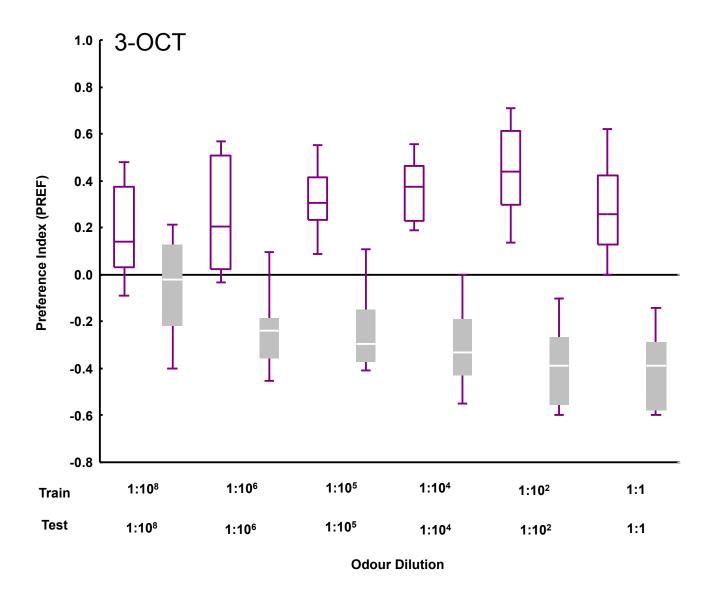


Fig: S1C

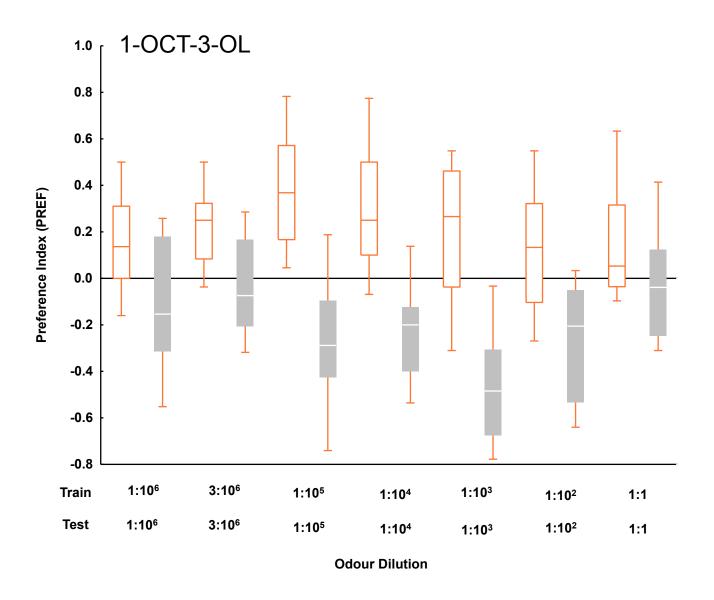


Fig: S1D

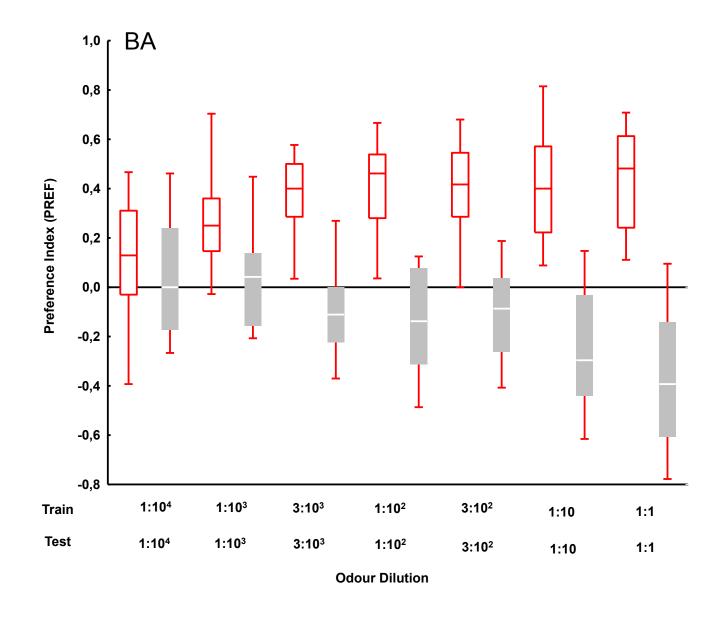


Fig: S2A (i)

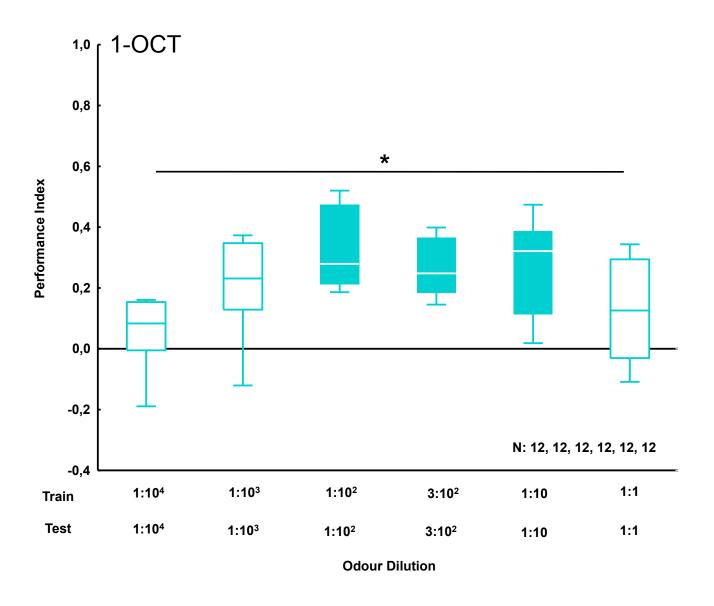


Fig: S2A (ii)

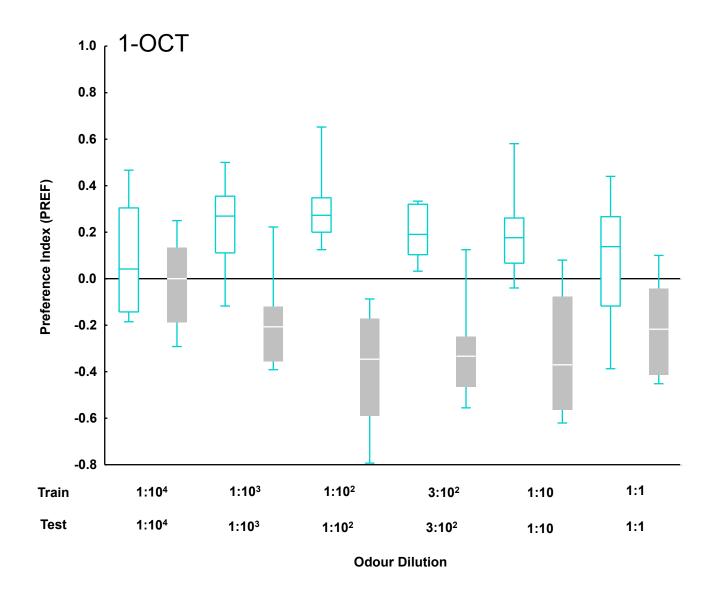


Fig: S2B (i)

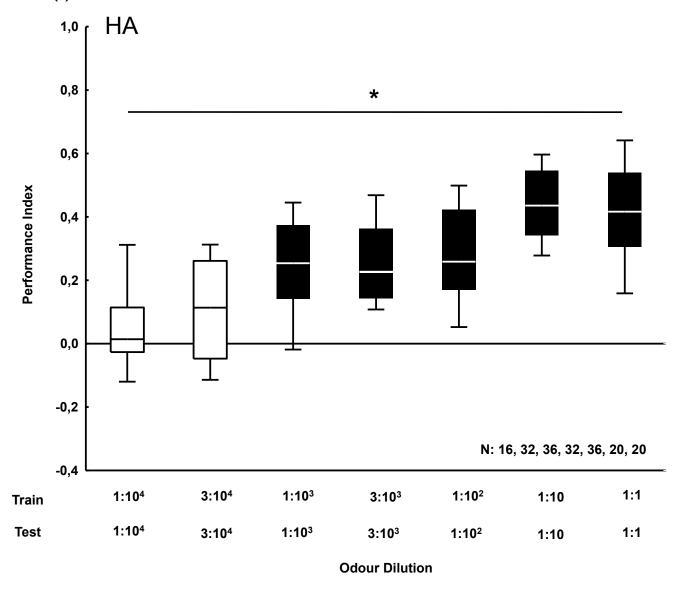


Fig: S2B (ii)

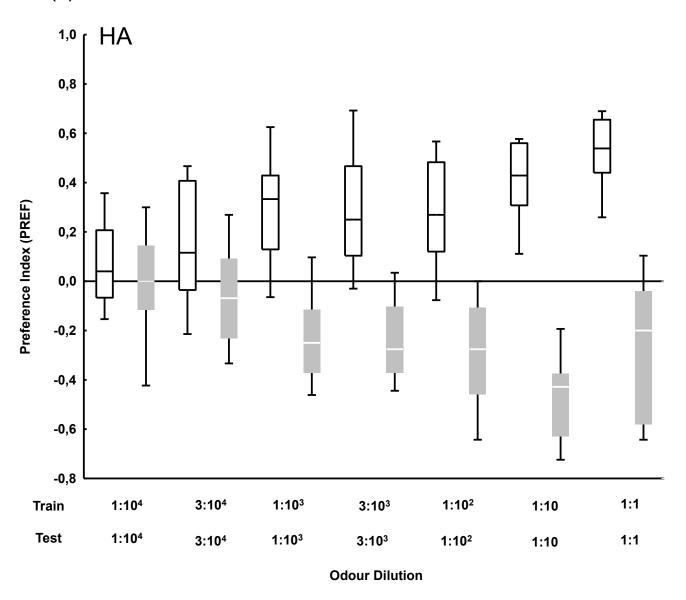


Fig: S2C (i) 1,0 | MCH 0,8 0,6 ns Performance Index 0,4 0,2 0,0 -0,2 N: 12, 12, 12 -0,4 Train 1:104 1:10² 1:1 Test 1:102 1:104 1:1

Odour Dilution

Fig: S2C (ii)

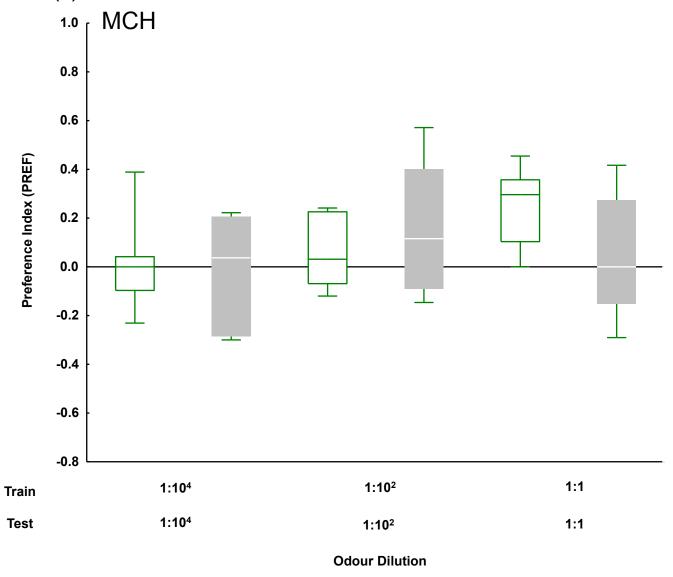


Fig: S2D (i)

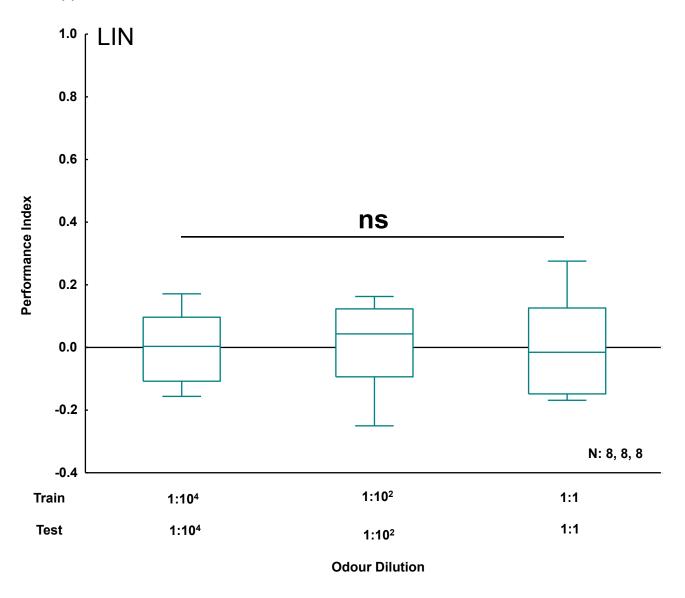


Fig: S2D (ii)

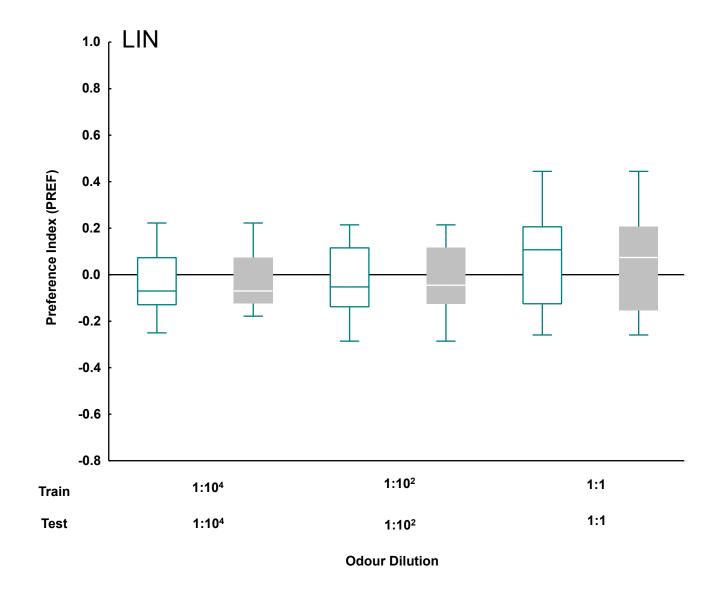


Fig: S3A

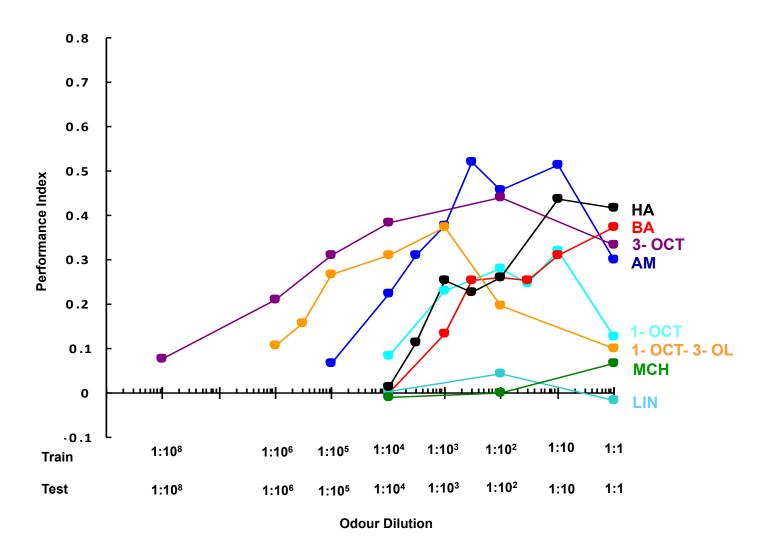


Fig: S3B

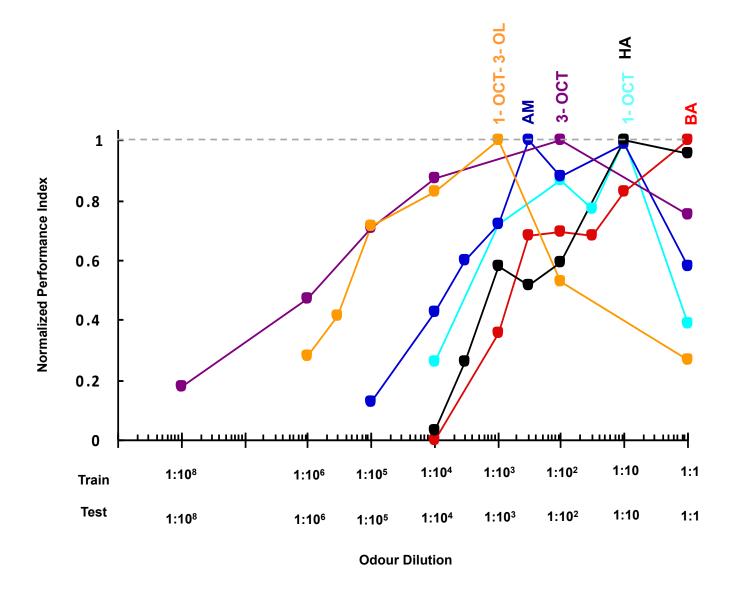


Fig: S4A

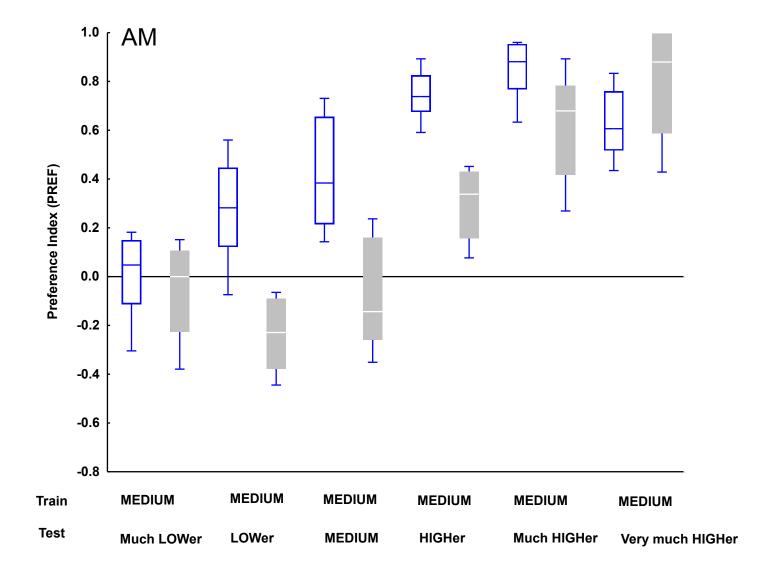


Fig: S4B

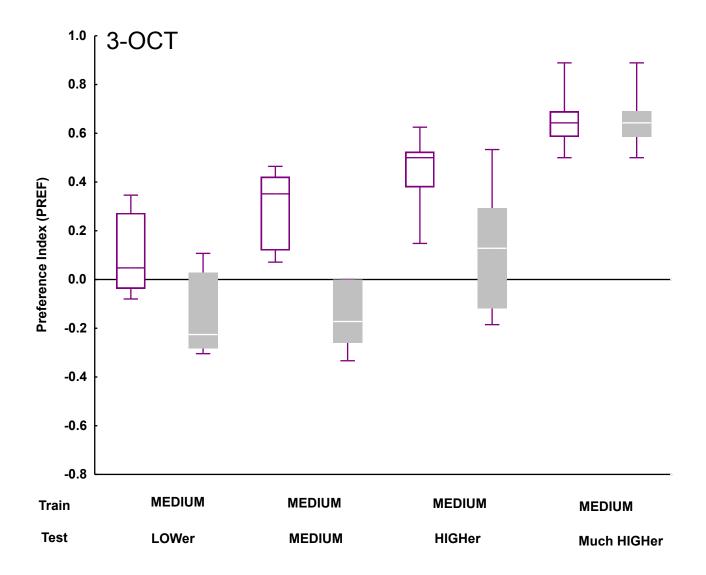


Fig: S4C

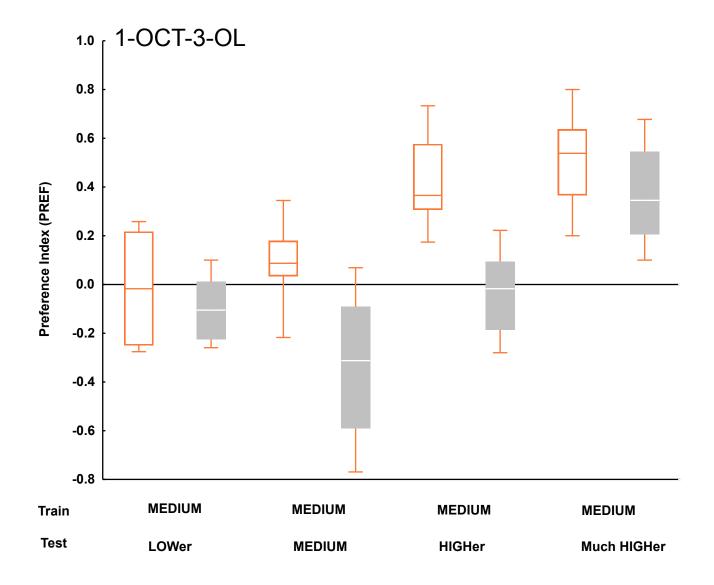


Fig: S4D (i)

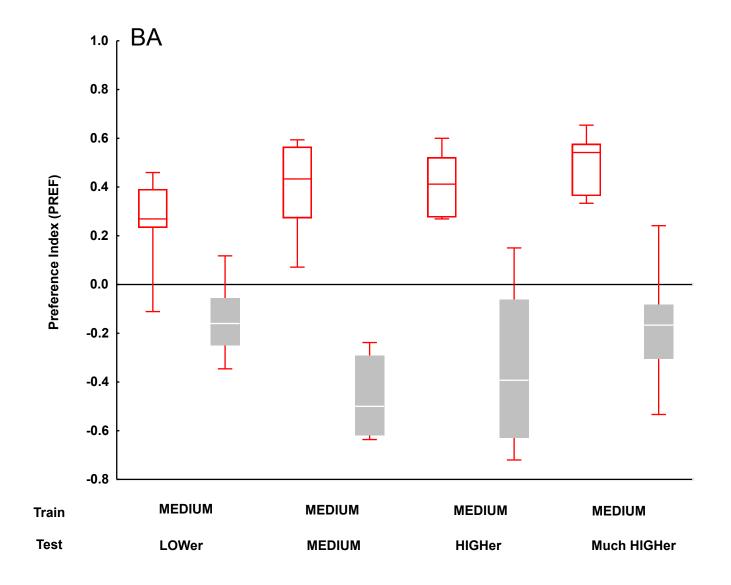


Fig: S4D (ii)

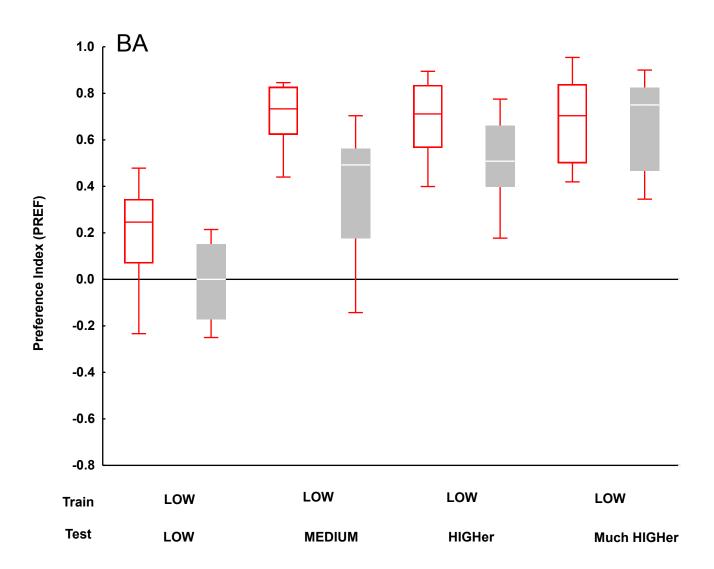


Fig: S5A

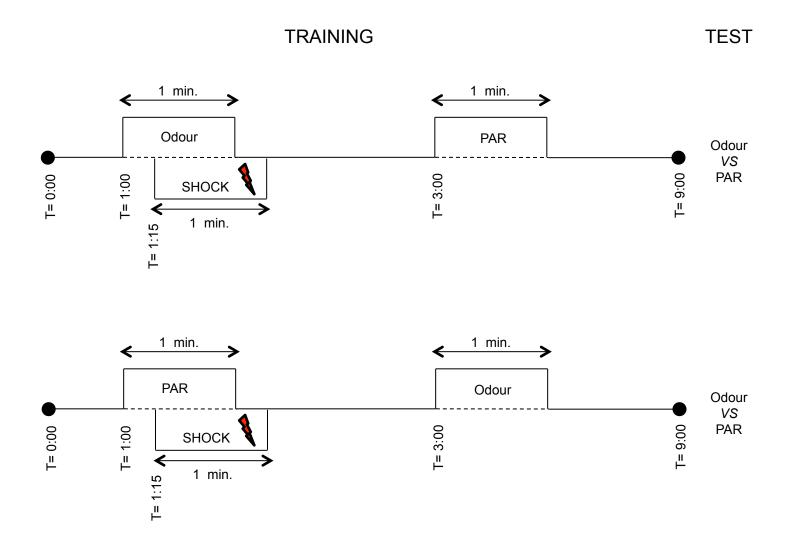


Fig: S5B

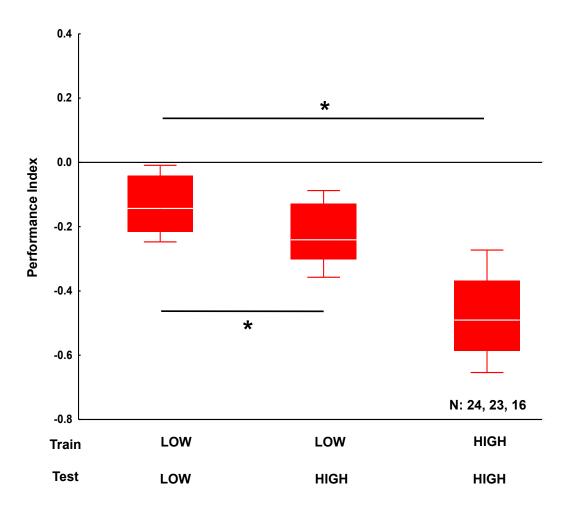
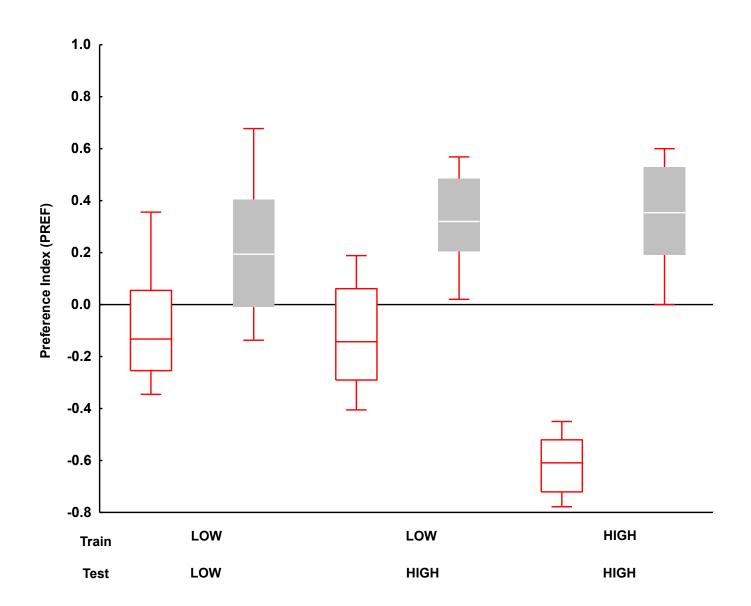


Fig: S5C



- **Fig. S1.** Preference scores for AM, 3-Oct, 1-Oct-3-ol and BA as related to Fig. 2. Preference scores underlying the associative performance scores in Fig. 2A–D. Preference is measured either after the odour was rewarded (e.g. AM+/EM, open boxes) or after the odour was not rewarded (e.g. AM/EM+, grey boxes). This is displayed in A–D for AM, 3-Oct, 1-Oct-3-ol and BA, respectively. Within each panel, preferences scores are plotted across the indicated dilution of the respective odour. Positive values indicate approach, negative values indicate avoidance.
- Fig. S2. Associative performance scores and the underlying respective preference values for 1-Oct, HA, MCH and Lin. (Ai) For 1-Oct we find an optimum function for associative performance scores across odour intensities. Performance scores at low (1:10³, 1:10⁴) and very at high (1:1) odour intensities are not significantly different from zero, whereas all other groups do show significant learning scores, indicated by filling of the boxes (OSS tests with P < 0.05/6 as criterion for significance). The KW test across groups yields H=18, d.f.=5, P<0.05. For this odour as well as the others displayed in this figure, we did not probe for intensity learning. (Aii) Preference scores of the two reciprocally trained groups (1-Oct+/EM, open boxes: 1-Oct/EM+, grey boxes) underlying the associative performance scores displayed in Ai. (Bi) At very low intensities of HA, performance indices are not significantly different from zero, whereas the other groups do show significant learning scores, indicated by filling of the boxes (OSS tests with P < 0.05/7 as criterion for significance). The groups are significantly different from each other (KW test H=60.1, d.f.= 6, P<0.05). (Bii) Preference scores of the reciprocally trained groups (HA+/EM, open boxes; HA/EM+, grey boxes) underlying the associative performance scores displayed in Bi. (Ci) For MCH, we do not find any appreciable associative performance scores across odour intensities, indicated by the lack of filling of the boxes (OSS tests at P>0.05/3); 'ns' refers to lack of between-group significance (KW test; H=4.1, d.f.=2, P>0.05), (Cii) Preference scores of the reciprocally trained groups (MCH+/EM, open boxes; MCH/EM+, grey boxes) underlying the associative performance scores displayed in Ci. (Di) For Lin, we do not find any appreciable performance scores across odour intensities, indicated by lack of filling of the boxes (OSS tests at P>0.05/3); 'ns' refers to lack of between-group significance (KW test: H=0.06, d.f.=2, P>0.05). (Dii) Preference scores of the reciprocally trained groups (Lin+/EM, open boxes; Lin/EM+, grey boxes) underlying the associative performance scores displayed in Di.
- **Fig. S3.** Semi-schematic summary of the dose–effect functions. (A) For eight different odours [*n*-amyl acetate (AM), 3-octanol (3-Oct), 1-octen-3-ol (1-Oct-3-ol), benzaldehyde (BA), 1-octanol (1-Oct), linalool (Lin), 4-methylcyclohexanol (MCH) and hexyl acetate (HA)], we plot the dose–effect curves of learnability, displaying odour dilution along the *x*-axis on a logarithmic scale and the median values of associative performance indices along the *y*-axis. (B) Same data as in A, normalized according to the highest median associative performance index obtained for the respective odour, excluding Lin and MCH, as they give no appreciable scores.
- **Fig. S4.** Preference scores for AM, 3-Oct, 1-Oct-3-ol and BA as related to Fig. 3. Preference scores underlying the associative performance scores in Fig. 3A–D. Preference is measured either after the odour was rewarded (e.g., AM+/EM, open boxes) or after the odour was not rewarded (e.g. AM/EM+, grey boxes). This is displayed in A–D for AM, 3-Oct, 1-Oct-3-ol and BA, respectively. Within each panel, preference scores for the respective odours are plotted dependent on the training–testing regime explained in the legend of Fig. 3. Positive values indicate approach, negative values indicate avoidance.
- Fig. S5. In an odour shock paradigm for adult *Drosophila* BA-memories are not intensity specific. In adult *Drosophila*, BA-memories have been reported to be not intensity specific as assayed in an odour-electric shock associative paradigm: higher-than-trained BA intensities support higher associative performance indices than the trained intensity [Yarali et al., 2009 (loc. cit. fig. 4D)]. The current experiment replicates this result. At 1-4 days after adult hatching, flies are collected in fresh food vials and maintained under culture conditions until they are used for experiments on the following day. Experiments are performed at 21–24 °C and 65–80% relative humidity, under white fluorescent light, in groups of ~ 50 . (A) Training starts (T=0.00 min) by loading the flies into the set-up as described in Schwaerzel et al. (Schwaerzel et al., 2003). The odour (benzaldehyde: BA; Merck Schuchardt OHG, Hohenbrunn, Germany; CAS: 100-52-7) is presented at 1:00 for 1 min and electric shock is applied from 1:15 on as 12 pulses of 96–100 V; each pulse is 1.2 s long and is followed by the next pulse with an onset-to-onset interval of 5 s. At time 3:00 min, a blank stimulus with the solvent (paraffin: PAR; Fluka, Steinheim, Germany; CAS: 8002-74-2) is presented for 1 min. After this BA-shock/PAR training, at 9:00 min, flies are transferred to a T-maze, where they are given the choice between one arm scented with the odour and the second arm supplied with PAR. After 2 min, the arms of the maze are closed and the numbers of flies (#) in the respectively scented arms are determined. A preference index (Pref) is calculated as: Pref = (#BA - #PAR)/#total. These Pref scores are documented in C. BAshock/PAR, represented by open red boxes. Another group of flies is trained reciprocally as PAR-shock/BA represented in C by filled grey boxes. Half the difference between the Pref values of these two reciprocally trained groups gives the associative performance index (PI): PI = $(Pref_{BA-shock/PAR} - Pref_{PAR-Shock/BA})/2$. The PI thus ranges from -1 to 1, negative values indicating conditioned avoidance from the odour (aversive learning), positive ones meaning conditioned approach towards the odour (appetitive learning). Half of the experiments were performed as explained above, whereas in the other half, flies were trained with the respectively reversed sequence (i.e. PAR/BA-shock or BA/PAR-shock) to balance for possible sequence effects. BA is diluted in PAR to the final concentrations given in B; in all cases, 250 µl of the BA solution or of PAR are placed in custom-made Teflon containers of 15 mm diameter. (B) Flies are trained and tested with either a Low (left; 1:10⁴) or with a high (right; 1:10³) BA intensity. In replication of Yarali et al. [Yarali et al., 2009 (loc. cit. fig. 3D)], these intensities support significantly different associative performance indices (MWU test: U= 18.0, P<0.05/2). Critically, we trained another group of flies with the low BA intensity and tested it with the high intensity (middle). This group, despite the training intensity not matching the test intensity, shows stronger associative performance scores than the group that was both trained and tested with the low intensity (MWU test: U=155.0, P<0.05/2). This replicates the result by Yarali et al. [Yarali et al., 2009 (loc. cit. fig. 4D)], confirming that BA-memories, at least in an odour shock paradigm using a single training trial, are not intensity specific. This contrasts with the intensity specificity of memory for 3-Oct, AM and MCH found by Yarali et al., (Yarali et al., 2009) in that paradigm, and with the intensity specificity of BA-memories in a larval odour sugar learning paradigm (see main text). (C) Documentation of the Pref scores underlying the associative performance indices in B.